# Multiple Sclerosis Pathology: Evolution of Pathogenetic Concepts

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**This historical review describes the evolution of pathogenetic concepts of multiple sclerosis (MS) from the viewpoint of pathology. MS research is based on studies of descriptive neuropathology, performed during the 19th and early-20th century, which defined the basic nature of the inflammatory demyelinating lesions. Advances in basic immunology and neurobiology, performed during the second half of the 20th century, paved the way for the understanding of the molecular mechanims involved in inflammation and well as tissue destruction in this disease. However, recent clinical and neuroradiological studies on the evolution of the disease and its brain lesions as well as ongoing attempts to define the genetic basis of the disease indicate that our current pathogenetic concepts may be too simple and that essential aspects of MS pathology have to be redefined.**

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Pathology is regarded as a medical discipline that describes in a detailed and objective way the alterations that distinguish diseased from normal tissue. Yet, even pathology is not immune against bias, as has been recently emphasized in an editorial on multiple sclerosis (MS) by Trapp (57), pointing out that "the eye only sees what the mind is prepared to comprehend." This problem becomes clearly evident when the historical development of MS pathology is reviewed. Although the substrate of interest—the tissue alterations in the central nervous system (CNS) of MS patients—has not changed over time, the focus of interest, the flavor of description (including its omissions) and the interpretation of the findings profoundly changed with time, heavily dependent upon current developments in other disciplines such as basic neurobiology, virology or immunology. Despite these problems, pathology in MS research fulfills an enormously important task: the critical reflection of whether new pathogenetic concepts coming from other research disciplines are compatible with the essential nature of the lesion in the involved target organ.

## THE GOLDEN CENTENIUM OF DESCRIPTIVE MS PATHOLOGY (1830-1930)

During this time the only feasible approaches to define the nature of the disease process in MS were anatomical and structural pathology. Thus, little bias was introduced into the field by other disciplines. Research was mainly driven by the development of new techniques for preparing and staining histological sections and by the evolution of knowledge regarding the anatomical and structural organization of the nervous system (29, 60).

Following the impressive documentation of gross anatomic changes in the CNS of MS patients by Carswell (10), Cruveilhier (15) and Valentiner (59), the first detailed account of the microscopic pathology of MS is generally ascribed to J. M. Charcot (12). In fact, his work summarizes the state of knowledge at his time in an excellent and educative manner. It was, however, not the first description of the essential microscopic pathological features of MS (see also 43). A fine example of earlier work is the publication by Eduard Rindfleisch, a Swiss pathologist, which was released in 1863 (48). Rindfleisch was the first to recognize that focal MS plaques are centered by small blood vessels. His work suggested that alterations of these vessels and the perivascular accumulation of "round cells" (now known to be mainly lymphocytes and macrophages) are essential features of early lesions. In passing, Rindfleisch also referred to the observation that "nerve fibres primarily lose their myelin and then can be traced a considerable distance into the connective tissue of the lesions as axons, devoid of sheaths." It is amazing that Rindfleisch was able to describe changes in axons and myelin, before classical silver impregnation techniques or myelin stains were developed (29, 60). The tools that made this possible were teased fiber and tissue preparations (48).

Charcot (11, 12) does, however, deserve the credit for putting all strings of research together into a concise disease concept. This is particularly impressive with regard to Charcot's attempts to correlate the locations as well as the structural features of MS lesions with clinical deficits observed in the patients. In addition, in comparison to previous work, the art of graphic documentation of structural changes in the nervous system had reached a new level of excellence in the French school, which allowed readers outside the narrow field of pathology to grasp the meaning of the pathological description. Examples of this artistic documentation are given in Figure 1.

Following Charcot's account of MS pathology, many studies appeared, extensively describing the structural changes in MS lesions. Fine structural changes of myelin were splendidly illustrated by Babinski (3), showing the segmental nature of the demyelinating process, the preservation of axons, as well as the close association between demyelination and inflammatory cells; in particular macrophages, which have taken up fragments of the degenerating myelin (Figure 1c). Another feature, which is illustrated in the study of Babinski, is the presence of axons, surrounded by very thin myelin sheaths with short internodes. This aspect was emphasized in more detail 20 years later by Otto Marburg (40). Marburg compared these myelin changes with those already known in the peripheral nervous system and first suggested that they may represent attempts of remyelination. It is interesting to note that these thin myelin sheaths were only seen after impregnation of tissue sections with osmic acid; a technique that decades later became a major

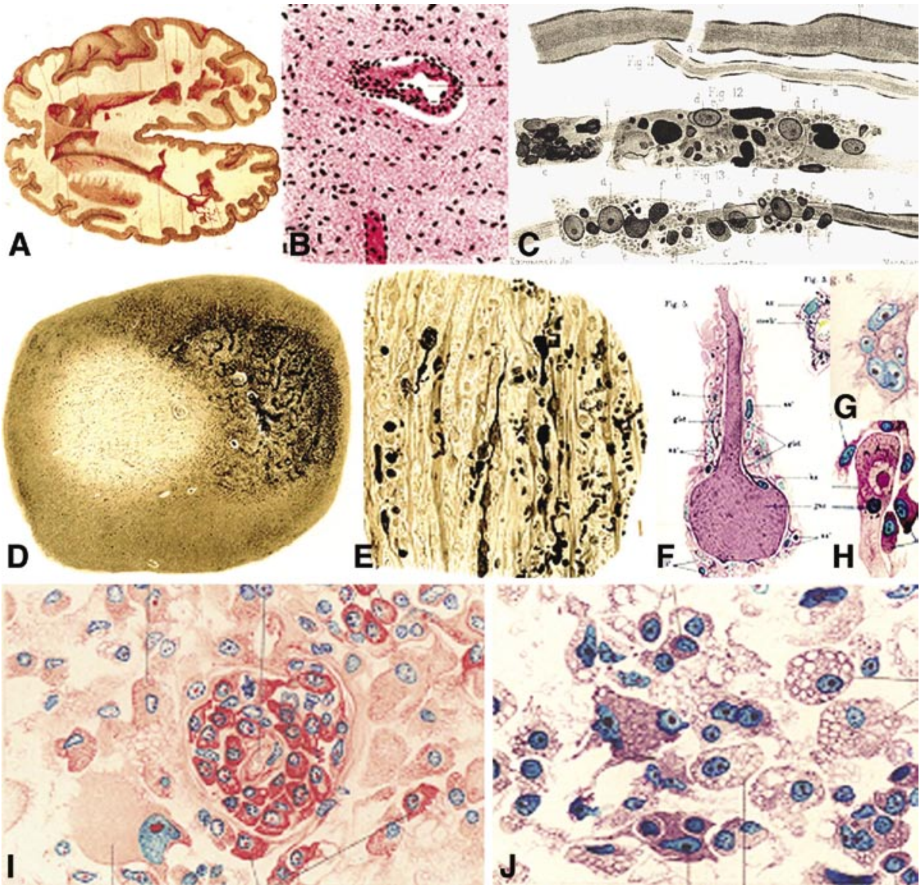


**Figure 1. A.** Macroscopic illustration of MS pathology given by Charcot (11); the illustration shows focal white matter lesions and their relation to blood vessels. **B, C.** Illustrations from Babinski (3); (**B**) documents the presence of perivascular inflammatory infiltrates and (**C**) the fine structure of myelinated fibers in the process of demyelination. **D, E.** Illustrations provided by Marburg (40); (**D**) shows an inactive demyelinated lesion with an adjacent active plaque with "Körnchenzellen" (macrophages with degradation products; (**E**) depicts active destruction of myelin sheaths, interpreted as a reflection of soluble "toxins", which destroy the myelin. **F, H.** Illustrations from Fraenkel and Jakob (24); (**F**) shows an axonal end bulb with an active lesions; (**H**) illustrates the close apposition of inflammatory cells (including plasma cells) to nerve cells. **G, I, J.** Illustrations from Jakob (33); (**G**) shows a multinucleated glia cell (astrocyte); (**I**) depicts the inflammatory reaction with multiple plasma cells and (**J**) shows the inflammatory infiltrate, composed of lymphocytes and macrophages as well as the glial reaction with bizarre multinucleated glial cells, which also may contain small round cells.

breakthrough for ultrastructural analysis of myelin.

It is clear from these early pathological descriptions that MS is not only a disease of myelin. As defined by Marburg (40), MS was regarded as a demyelinating disease with relative preservation of axons, but he also pointed out that the emphasis has to be laid on the term "relative" and that all MS lesions show at least some degree of axonal injury and destruction. Axonal pathology was the focus of interest between 1880 and 1930 (for details, see 35). Axonal loss was found to vary from case to case and in different lesions and an extensive axonal loss was associated with severe and persistent clinical deficit (11). Acute axonal injury, such as the formation of spheroids and axonal end bulbs, was found mainly to be a feature of early and active lesions (18, 24; Figure 1F) and was associated with macrophages, found in close contact to them (24). Other studies also described attempts of axonal regeneration and sprouting (18, 33). Finally, it was noted that axonal loss in the plaques may result in secondary Wallerian degeneration in the respective tracts (1, 40).

Other studies focused on inflammation and the patterns of glia reaction. As mentioned above the presence of round cell infiltrates and the involvement of macrophages in the demyelinating process has been already noted in the earliest studies by Rindfleisch (48), Charcot (12), Babinski (3; Figure 1B) and Marburg (40), and even the presence of plasma cells was well documented (33; Figure 1H, I). In addition, it was noted that glia cells, in particular the astrocytes, were also highly abnormal. Multinucleated process bearing cells, later identified as astrocytes, were first depicted by Rindfleisch (48). More detailed accounts of astrocytic pathology were given by Anton and Wohlwill (1) and Jakob (33). They described in early active lesions very large and bizarre astrocytes containing multiple and sometimes fragmented nuclei (Figure 1G, J) or engulfing other cells (Figure 1J).

Other very important papers from this period dealt with lesion topography in the brain and spinal cord (11, 23, 54) and its relation to clinical disease. In addition, variants of MS were defined, such as Marburg's acute MS (40), Devic's type of neuromyelitis optica (17) and Balo's concentric sclerosis (4). It was also noted that demyelinated lesions are not restricted to the white matter, but can also affect the cortex and grey matter in the brain stem (7).

Despite all these detailed descriptions, 2 main questions remained controversial. The first relates to the inflammatory nature of the disease. Although the presence of inflammatory infiltrates was widely recognized, it remained controversial as to whether this was a primary or a secondary event. Although most authors favored the former, an example of the latter hypothesis comes from the work of Müller (42), who saw MS as a primary disease of astrocytes. Interestingly, the question of the primary nature of inflammation in MS is still controversial today (5). The second controversial aspect was the mechanism of tissue injury. While many studies favoured a cellular attack on myelin and axons, mainly performed by macrophages (3; Figure 1C), others postulated the presence of soluble toxins, that enter the brain through leaky vessels and directly destroy the myelin sheaths (40, Figure 1D, E).

## NEW DEVELOPMENTS IN NEUROIMMUNOLOGY AND NEUROBIOLOGY DRIVE MS RESEARCH IN THE SECOND HALF OF THE 20TH CENTURY

The first description of an inflammatory demyelinating disease, induced by active sensitization of susceptible animals with brain tissue (49), introduced a major change in the focus of pathological MS research. In subsequent years it turned out that this autoimmune disease of the CNS is mainly driven by a T-cell mediated immune response directed against myelin antigens. Based on the close similarities between the demyelinating plaques in MS patients with those found in chronic models of experimental autoimmune encephalomyelitis (EAE, 37), the view that MS is a T-cell mediated autoimmune disease became a dogma.

Undoubtedly, EAE research was fundamental in developing the concept of organ specific autoimmunity and paved the way for our understanding of basic mechanisms of brain inflammation. Tools to identify specific leukocyte subsets became available, and they were immediately used to characterize the nature of the inflammatory reaction in MS. These studies were made possible by the development of new immunocytochemical techniques, headed by the discovery of the peroxidase/anti-peroxidase technique by Sternberger in 1970 (55; Figure 2).

It became clear that the dominant inflammatory cells in MS lesions are T-cells and macrophages with a lower and variable contribution of B-cells and plasma cells (19, 44; Figure 2A-C, G-H). An early and frequently quoted study described that Class II MHC restricted CD4 positive cells dominate in active lesions (58). This view fitted the immunological dogma very well; that autoimmune encephalomyelitis is a disease which is mediated by Class II MHC restricted Th1 cells, and thus, was highly appreciated by experimental immunologists. Yet, at the same time—and also in later studies—a dominance of Class I MHC restricted CD8 positive T-lymphocytes in all MS cases and lesion stages was shown (6, 27; Figure 2G), but largely ignored by the community of MS researchers. Only recently, when a dominant clonal expansion of CD8 positive cells in MS brains was documented (2), has the importance of Class I restricted T-cells in MS lesions received broader acceptance.

Experimental studies outlined the pathways and mechanisms of leukocyte migration into inflammatory (brain) lesions (39). Adhesion molecules, expressed on the surface of endothelial cells and leukocytes have been found essential for inflammatory cells to leave the circulation. This process is further strengthened by chemokines, which are produced within the tissue and can attract specific leukocyte subsets into

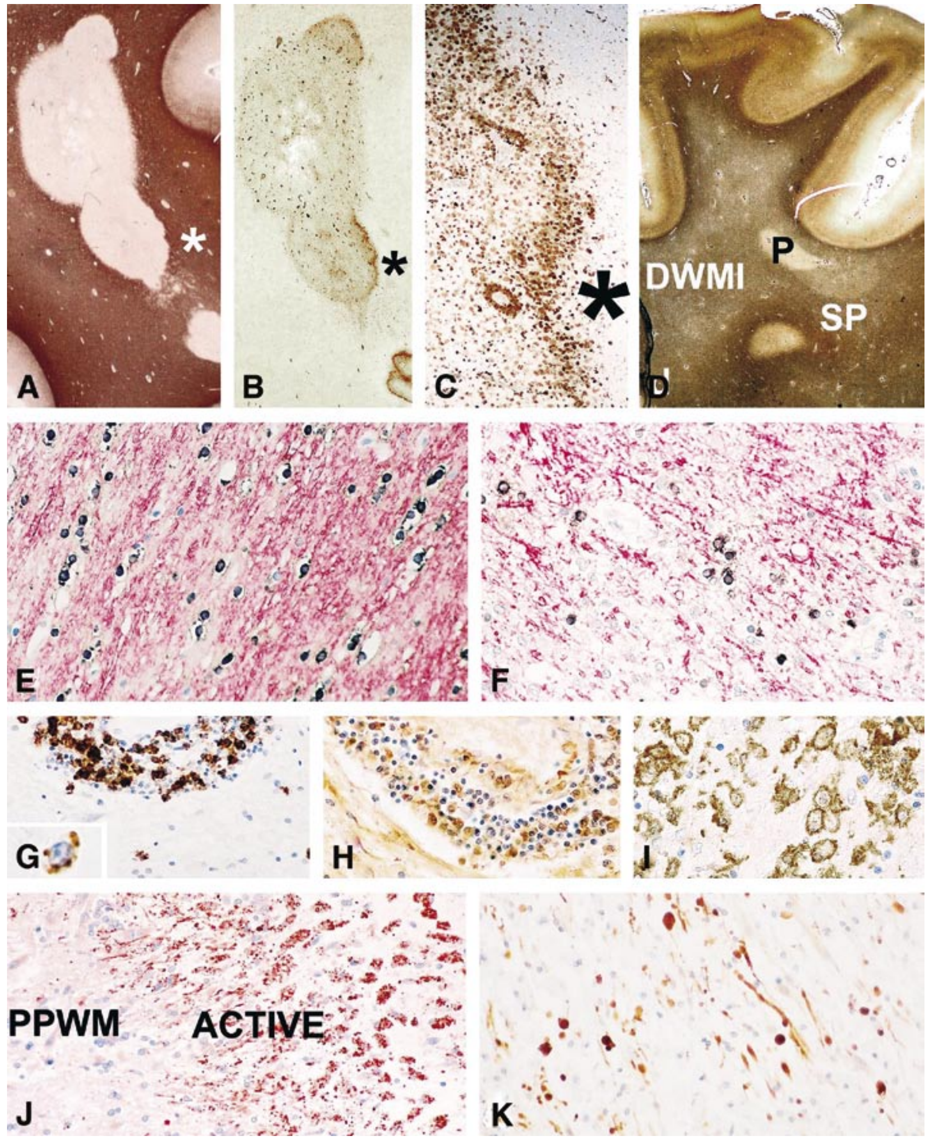


**Figure 2. A-C.** Actively demyelinating plaque in acute multiple sclerosis shows loss of myelin (**A**) and the accumulation of macrophages at sites of ongoing myelin destruction (**B, C**); The asterisks label the position of (**C**) within the plaque; (**A**) immunocytochemistry for myelin oligodendrocyte glycoprotein; ×3.5. **B, C.** Immunocytochemistry for CD 68 (macrophages); **B:** ×3.5; **C:** ×30. **D.** Different types of white matter pathology in secondary progressive MS; one focal demyelinated plaque (P), a sharply demarcated remyelinated shadow plaque (lesion above SP) as well as diffuse, poorly demarcated white matter injury (DWMI); Bielschowsky silver impregnation; ×2.5. **E, F.** oligodendrocytes in the normal white matter (**E**) and at the edge of a demyelinated plaque (**F**) in acute MS; oligodendrocytes are reduced in density at the plaque margin and within the plaques; in situ hybridization of proteolipid protein mRNA (black) and immunocytochemistry for proteolipid protein (red); ×300. **G-I.** Inflammation in MS lesions; (**G**) Class I MHC restricted CD8 positive T-cells; insert shows a cytotoxic T-cell with cytoplasmic granzyme B reactivity; (**H**) immunoglobulin containing plasma cells within a perivascular cuff; (**I**) macrophages within an active lesions; immunocytochemistry for CD8 or granzyme B (**G**), immunoglobulins (**H**) and CD 68 (**I**); ×300. **J.** Active MS lesion, reactive for complement on degenerating myelin and within myelin degradation products; immunocytochemistry for C9neo antigen; ×200. **K.** acute axonal injury in an actively demyelinating MS lesion, showing abundant axonal spheroids and end bulbs; immunocytochemistry for phosporylated neurofilament; ×500.

the inflammatory focus through the interaction with their specific receptors. Finally, proteases are necessary to allow the passage of inflammatory cells through the vascular barrier (16). The respective molecules have subsequently been identified within active MS lesions (9, 16, 30) and became or are currently being developed as targets for anti-inflammatory therapy (41).

Other studies focused on the expression of immune related molecules in MS lesions. Not surprisingly, considering the inflammatory nature of the disease, many of these molecules were found, but so far no pattern has become apparent, which distinguishes MS from other T-cell mediated inflammatory brain diseases. The immune related molecules include proteins involved in antigen-specific T-cell activation (histocompatibility antigens, co-stimulatory molecules), in immune regulation (cytokines) or in immune mediated tissue injury (cytokines or other toxic effector molecules and complement; Figure 2J). Furthermore, with the exception of Type I interferons, which are currently used for immunomodulatory treatment (31), none of these molecules so far were found useful as diagnostic or prognostic markers in the blood and cerebrospinal fluid of MS patients or survived as targets for effective therapy. The reasons for this unsatisfactory situation may be manifold. Inflammation and immune mediated tissue injury is accomplished by many different and frequently redundant mechanisms. The presence of a certain immune effector molecule within a lesion allows only limited conclusions regarding its importance in the process of tissue injury. Furthermore, the majority of such immunopathological studies in MS were based on very small numbers of cases, the staging of disease and lesions was frequently inadequate, and the interpretation of the findings was at least in part biased by current immunological concepts, mainly derived from studies of EAE. These points are important, since recent studies indicate that the immunopathology may be heterogeneous between different MS lesions and in particular between lesions from different patients (38).

A second major boost of pathological MS research came from the field of neurobiology in the 1960s, when the fine structural organization of myelin, its relation to oligodendrocytes and the processes of myelination and remyelination were elucidated by electron microscopy and tissue culture studies (8). The application of this knowledge to MS lesions was systematically pursued, in particular by John Prineas. By combining ultrastructural work on excellently preserved MS tissue with state of the art immunocytochemical methods, he clearly defined patterns of myelin destruction and the fate of oligodendrocytes within the lesions (45; Figure 2E, F). He also substantially contributed to our knowledge regarding endogenous remyelination in MS lesions (45-47). Furthermore, it became clear that shadow plaques—originally named "Markschattenherde" by Schlesinger in 1909 (52; Figure 2d) and interpreted as areas of incomplete demyelination—were, in fact, remyelinated plaques (37, 45).

The importance of the different concepts of immunology and autoimmunity research brought to the understanding of MS is undisputed. All anti-inflammatory and immunomodulatory treatments currently applied in MS patients are based on this background. However, there is also a back side of the coin. Nearly by dogma, MS became an autoimmune disease, and myelin was believed to be the target, although alternative explanations for the pathogenesis of MS were propagated and systematically followed. Virologists tried hard to identify "the MS virus" and this is documented by multiple claims in the literature. Unfortunately no such claim turned out hard enough to be universally accepted by the research community. However, this does not rule out that MS may be triggered or even propagated by an infectious agent (28).

Unfortunately the stringent pathogenetic concept of MS, being an autoimmune process directed against myelin, clouded the view on the whole picture of the disease. As mentioned above, axonal pathology (Figure 2K)—although well defined in early studies on MS pathology—has vanished from the focus of interest for many decades. In addition, research focused nearly exclusively on structural alterations within focal demyelinated plaques, although many clinical studies suggested that neurological deficit in MS patients can not solely be explained by the size and distribution of the plaques (32, 51). Apparently, essential aspects of the spectrum of MS pathology, such as axonal involvement, cortical and grey matter pathology, as well as diffuse alterations in the normal appearing white matter, escaped attention during this period.

## MAGNETIC RESONANCE IMAGING RAISES NEW AND FUNDAMENTAL QUESTIONS OF MS PATHOLOGY (1980-PRESENT)

When magnetic resonance imaging (MRI) and spectroscopy (MRS) were introduced in routine neurological practice, it was considered to be just a useful tool to visualize focal white matter plaques, and consequently, to support differential diagnosis and provide outcome measures in clinical trials. Yet, MRI and MRS accomplished much more. Repeated scanning, designed in a prospective way, gave insights into the dynamic evolution of pathological changes and provided 3-dimensional quantitative information on a large scale of patients. In addition, MR spectroscopy provided some insight into specific molecular changes within the tissue. For MS research it was fortunate that one of these molecules easily detected by MRS, N-acetyl aspartate (NAA), turned out to be a specific and sensitive marker for neuronal and axonal injury (53). The prominent reduction of NAA within the lesions, but also within the apparently normal white matter (25), forced neuropathologists to reinvestigate the problem of axonal pathology in this disease. In these studies most of the aspects, described during the first decades of the 20th century were confirmed. However, in addition they provided insight into the quantitative dimension of axonal loss (21, 36, 56) and emphasized the importance of Wallerian tract degeneration in relation to global alterations within the normal appearing white matter (20, 26).

For other recently observed MRI alterations, however, a pathological substrate is still missing. Imaging studies clearly show that MS is not only a disease with multifocal lesions in the brain, but that the disease process apparently affects the CNS in a diffuse and global manner. In other words there are major abnormalities in the normal appearing white matter, which are associated with progressive atrophy of the white and grey matter (13, 50). Interestingly, these alterations can only be partly explained on the basis of tissue destruction within focal plaques. Furthermore, brain atrophy affecting both the grey and white matter starts very early in the disease course and can be detected even in patients presenting with the first bout of the disease. So far the normal appearing white matter has largely been neglected by neuropathologists.

Another intriguing finding, coming from prospective serial MRI studies, is that weeks before new (gadolinium enhancing) focal lesions appear in the brain, there are subtle focal changes within the white matter just at the location, where the plaques will appear at later stages (22). These findings suggest that a focal latent disease process precedes the formation of classical plaques. What the nature of this process is will have to be clarified by neuropathology in the future.

## FUTURE OUTLOOK

As outlined above there are many open questions in MS research that need to be addressed by pathological analysis. Considering the profound heterogeneity between different plaques from the same (and even more from different patients [38]), as well as the polygenic nature of the disease (14), broadly designed studies to correlate the genotype of the patients with the pathological phenotype of the respective lesions will be instrumental. Fortunately, genotyping turned out to be possible in archival pathological material, even in patients who died years or decades earlier. In addition, gene profiling of well defined tissue will also become more important. Due to the heterogeneity of tissue alterations between lesions, or even within a given lesion, such profiling will have to rely on material that is accurately classified regarding its pathological phenotype. Newly developed microdissection techniques, together with very sensitive methods of mRNA detection, allow profiling of gene expression in defined tissue areas or even single cells. It is hoped that further improvement of such techniques will make them applicable even in archival fixed and embedded autopsy material.

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